Rac1 and Rac3 Are Targets for Geranylgeranyltransferase I Inhibitor-Mediated Inhibition of Signaling, Transformation, and Membrane Ruffling

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ABSTRACT

Rac1, a Rho family GTPase, is a mediator of diverse cellular functions including membrane ruffling, cell cycle progression, and transformation. Rac3, a close relative of Rac1, is less well characterized. Posttranslational addition of geranylgeranyl isoprenoid lipids to Rac proteins is required for biological activity. Inhibitors of geranylgeranyl transferase I (GGTIs) are currently under investigation as a possible anticancer therapy, although the targets of GGTIs have not been determined. We created COOH-terminal mutants of Rac1 and Rac3 that are farnesylated and used them to characterize Rac1 and Rac3 as physiological targets of GGTIs. We show that, like Rac1, activated Rac3 causes transformation and leads to membrane ruffling. Farnesylated versions of Rac1 and Rac3 retain the ability to signal to the transcription factor c-Jun and cause membrane ruffling and transformation, indicating that switching isoprenoid modification does not alter function. Finally, treatment with GGTIs led to the inhibition of membrane-ruffling and transforming activities of both activated and wild-type Rac1 and Rac3. However, the farnesylated versions of both activated and wild-type Rac1 and Rac3 were resistant to the inhibitory effects of GGTIs. These results illustrate that Rac1 and Rac3 are potential physiological targets for these novel drugs.

INTRODUCTION

The small GTPases Rac1 and Rac3 are members of the Rho family. Like Ras proteins, Rho family proteins cycle between active GTP-bound and inactive GDP-bound states (1–3). They are positively regulated by Dbl family guanine nucleotide exchange factors, which regulate the Rac GTPases, are substrates for GGTase I and are logical targets for GGTIs as anticancer agents, we created COOH-terminal mutants of WT and activated forms of Rac1 and Rac3 that render them exclusively geranylgeranylated, farnesylated, or UN (no isoprenoid group is added). These mutants were used to characterize the sensitivity of Rac1 and Rac3 to GGTIs and FTIs in signaling, membrane ruffling, and transformation. Our results suggest that, in contrast to what has been reported for RhoA (27), Rac1 and Rac3 are sufficient to mediate the inhibitory effects of GGTIs on transformation and membrane ruffling and appear to be potential physiological targets for this class of drugs.

MATERIALS AND METHODS

Prenylation Mutants of rac1 and rac3. Human WT rac1 and rac3 in the vector pcDNA3.1+ were obtained from the Guthrie cDNA Resource (Sayre, PA). A Q61L mutation was introduced into Rac3 by the QuickChange Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. All primers were generated by the Nucleic Acids Core Facility at the University of North Carolina at Chapel Hill. The Rac1 Q61L construct has been described previously (11). The CAAX motifs of human Rac1 and Rac3 in either WT or oncogenically activated versions (12V or 61L) were mutated by PCR by methods previously published for Ras family member proteins (28). Additionally, a G12V mutation was created within the S12N primer for rac3. Primers at the 3’ ends were designed to alter the CAAX motif of Rac1 from the parental CLLL (X = L; GG) to CVLS (X = S; F), and SLLL (C to S; not prenylated, UN) and to alter the CAAX motif of Rac3 from the parental CTVF (X = F; possibly GG or F) to CTVF (X = L; GG), CVTS (X = S; F), and STVF (C to S; UN). PCR products of rac1 and rac3 were digested with BamHI (all restriction enzymes; Invitrogen, Carlsbad, CA) at the sites intro-
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duced at the 5′ and 3′ ends and ligated in-frame with the HA epitope tag into the vector pCGN-hyg for expression in mammalian cells (28). PCR products were also digested with BamHI and EcoRI and ligated in-frame with an EGFP tag into the mammalian vector pEGFP-C1 (Clontech, Palo Alto, CA), which was cut previously with BglII and EcoRI. Expression plasmids were thus generated in pCGN and in pEGFP-C1 for each of the following: rac1WT (WT) or 61L)-P (CAAX = CLLL); rac1WT (WT) or 61L)-F (CVLS); rac1WT (WT) or 61L)-UN (SLLL); rac3WT (WT, 12V or 61L)-P (CTVF); rac3WT (WT, 12V or 61L)-GG (CTVL); rac3WT (WT, 12V or 61L)-F (CTVS); and rac3WT (WT, 12V or 61L)-UN (STVF). All mutations and ligation junctions were verified by forward and reverse sequencing.

Cell Culture and Transfections. NIH 3T3 mouse fibroblasts were grown in DMEM (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% calf serum (Life Technologies, Inc.) and 1% P/S (complete medium) and maintained in 10% CO₂ at 37°C. Cells were plated the day before transfection at 5 × 10⁵ cells/100-mm dish, 2.5 × 10⁵ cells/60-mm dish, or 1 × 10⁵ cells/35-mm 6-well plate. NIH 3T3 cells were transfected by calcium phosphate coprecipitation for 3–5 h followed by glycerol shock for 3 min as described previously (28) or with LipofectAMINE and Plus reagents (Invitrogen) following the manufacturer’s instructions. Stable cell lines were created in NIH 3T3 cells after transfection with 200 ng of pCGN-hyg constructs expressing the Rac1 and Rac3 prenylation mutants. Two days after transfection, one-third of the cells were split into complete medium containing 200 μg/ml hygromycin B (Roche, Indianapolis, IN) in 100-mm dishes. Cells were maintained in hygromycin B for 10–12 days, after which colonies were pooled for use. To prevent loss of protein expression, stable cell lines were maintained continuously in hygromycin B until they were split for experiments.

Swiss 3T3 mouse fibroblasts, generously provided by Krister Wennerberg and Keith Burridge (University of North Carolina at Chapel Hill), were grown in DMEM supplemented with 10% fetal bovine serum (HyClone, Logan, UT) and 1% P/S and maintained in 10% CO₂ at 37°C. Cells were plated as described above for NIH 3T3 cells. Transfections were carried out out with FuGENE 6 (Roche) according to the manufacturer’s instructions.

Western Immunoblotting. Stable cell lines were plated on 60-mm dishes and allowed to grow for 2 days. Cells were lysed in 300 μl of TX-100 lysis buffer [50 mM Tris (pH 7.5), 100 mM NaCl, 1% (v/v) Triton X-100, 5 μg/ml aprotinin, 10 μM leupeptin, 20 mM β-glycerophosphate, 12 mM p-nitrophenylphosphate, and 0.1 mM sodium vanadate]. Lysates were cleared by centrifugation at 12,000 rpm for 10 min at 4°C, and protein concentration was determined with a colorimetric assay (Bio-Rad, Hercules, CA). Samples were prepared in 5× Laemmli sample buffer, and 20 μg of protein from each sample were run on 15% SDS-PAGE gels. Proteins were transferred to polyvinylidene difluoride (Immobilon-P; Millipore, Bedford, MA). Membranes were incubated for 1 h in 20% methanol:acetic acid, and stained with 0.4% crystal violet in 20% ethanol. Stained foci were then counted for quantification of transforming activity.

For soft agar assays, NIH 3T3 cells stably expressing pCGN-hyg vector, pCGN-rac1(61L)-F (WT), or pCGN-rac1(61L)-P (WT) were plated in complete medium containing 0.4% agar and DMSO vehicle, 10 μM FTI-2153, or 10 μM GGTI-2166 on top of a bottom layer of complete medium containing 0.6% agar. Colonies were allowed to form for 14–21 days, after which they were photographed under the ×4 objective.

Localization Assays and Fluorescent Microscopy. NIH 3T3 cells were plated on glass coverslips in 35-mm, 6-well plates. For visualization of localization and subcellular distribution of the lamellipodia and membrane ruffles, cells were transiently transfectsed with 1 μg of pEGFP-C1 vector, pEGFP-rac1(61L)-F, or pEGFP-rac3(61L)-F prenylation mutants [Rac1(61L)-WT, -F, Rac1(61L)-P, or Rac1(61L)-GG] and 125 ng of pβ-luc, a c-Jun luciferase reporter construct (a gift of Silvio Gutkind; NIH, Bethesda, MD). All transfections were performed in duplicate. Cells were placed in DMEM containing 0.5% calf serum containing either DMSO vehicle or 1 μg GGTI-2166 immediately after glycerol shock and were grown for 20–24 h. The cells were then rinsed with 1× PBS and lysed in 1× lysis buffer (Amersham Biosciences), and luciferase activity was measured with enhanced chemiluminescence reagents (Amersham Biosciences) in a Monolight 2010 luminoimeter (Analytical Luminescence, San Diego, CA).

RESULTS

Both Geranylgeranylated and Farnesylated Forms of Rac1 and Rac3 Are Expressed Equivalently in NIH 3T3 Cells. To validate the use of the prenylation mutants in the experiments shown, it was

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first necessary to determine their expression levels. Constructs were made to express Rac1 with a parental CAAX motif, which should be geranylgeranylated, or a mutated CAAX motif that made it either F or UN. Due to the possibility that the parental CAAX motif of Rac3, CTVF, could be a target for either GGTase I or FTase (26), constructs were made to express Rac3 with the parental CAAX motif or a mutated CAAX that made it exclusively G, exclusively F, or UN. As shown in Fig. 1A, all prenylated mutants were expressed in stable cell lines at levels approximately equivalent to that of the Rac proteins with the parental CAAX motifs. Interestingly, Rac3-UN was expressed at significantly higher levels than any other Rac3 protein (Fig. 1A). This held true in at least six independently isolated cell lines. Although the basis for the differential expression is not clear, expression of Rac3-UN appeared to enhance cell viability, as reflected in a much larger number of colonies obtained from antibiotic selection for Rac3-UN compared with any other Rac3 protein (data not shown).

Geranylgeranylated Forms of Rac1 and Rac3 Show Dose-Dependent Sensitivity to GGTIs. To determine whether geranylgeranylated forms of Rac1 and Rac3 are sensitive to GGTIs, cells transiently expressing activated Rac1, Rac3, or Rac3-GG were treated with increasing doses of GGTI or with FTI. Cells were crudely fractionated into cytosolic S100 and membrane-containing P100 fractions. Fig. 1B shows that with increasing doses of GGTI, Rac1, Rac3, and Rac3-GG proteins all decrease within the membrane fraction (P100) and increase within the cytosolic fraction (S100), as shown by the decrease in the P:S ratio from DMSO-treated samples to those treated with increasing doses of GGTI. Loss of protein from the membrane fraction indicates loss of prenylation because prenylation is necessary to anchor Rho family proteins in the membrane (18). Comparison of the P:S ratio of GGTI-treated samples with the P:S ratio of the DMSO-treated samples shows that for Rac1, 67%, 87%, and 97% of the protein was moved to the cytosolic fraction after treatment with 1, 5, and 10 μM GGTI, respectively. For Rac3, 69%, 75%, and 83% was moved at 1, 5, and 10 μM GGTI, respectively, and for Rac3-GG, 87%, 78%, and 89% was moved at 1, 5, and 10 μM GGTI, respectively. Conversely, with 10 μM FTI treatment, the amount of Rac1, Rac3, or Rac3-GG protein in the membrane (P100) fraction appears similar to the amount with DMSO treatment, indicating that prenylation of geranylgeranylated forms of Rac proteins is not sensitive to the inhibition of FTase by FTIs. When the percentages are compared, more protein was moved from the membrane to the cytosol for Rac1 than either Rac3 or Rac3-GG at the highest dose of GGTI, but there was also more protein in the P100 fraction compared with the S100 fraction for Rac1 than either Rac3 or Rac3-GG. This suggests there may be some differences between Rac1 and Rac3 in partitioning between the membrane and cytosol and/or in general sensitivity to GGTI treatment.

Both Geranylgeranylated and Farnesylated Forms of Rac1 and Rac3 Signal to c-Jun, but Only Signaling from the Geranylgeranylated Rac Proteins Is Sensitive to GGTIs. Activated Rac3 has been reported to act as a downstream target of JNK, c-Jun. We evaluated the ability of the prenylation mutants of activated Rac1 and Rac3 to signal to the c-Jun pathway in the presence or absence of GGTIs by using a c-Jun luciferase reporter assay. Fig. 2 shows that, as expected, both activated Rac1 and Rac3 can signal robustly to c-Jun, and both prenyl groups (GG or F) support signaling activity.

To determine whether the ability of Rac1 and Rac3 to transcriptionally transactivate c-Jun is sensitive to GGTI-mediated inhibition, we performed the reporter assays in the presence of 1 μM GGTI. Decreases in activation by Rac1, Rac3, and Rac3-GG (Fig. 2, A and B) demonstrate that all three are sensitive to GGTI. From Fig. 1B, we determined that 67% of Rac1, 69% of Rac3, and 87% of Rac3-GG has been moved from the membrane fraction at the dose of GGTI used, which may account for incomplete inhibition of c-Jun transactivation. Rac3 with the parental CAAX motif was just as sensitive to GGTI as Rac3 with a CAAX motif that should only be geranylgeranylated (Rac3-GG). This argues against the notion that the CTVF CAAX motif of Rac3 is normally a target for both GGTase I and FTase and suggests that the majority of Rac3 is indeed geranylgeranylated. The inhibition of the signaling activity of geranylgeranylated Rac1 and Rac3 suggests that they could be targets of GGTI activity. In contrast, the signaling activity of farnesylated Rac1 and Rac3 is not sensitive to GGTI.

Farnesylated and Geranylgeranylated Forms of Rac1 and Rac3 Are Both Morphologically Transforming. The similarity of Rac3 to Rac1 suggests that it has oncogenic potential, yet the transforming activity of Rac3 has not been demonstrated. It has been shown that activated Rac1 cooperates with activated Raf in focus formation assays (13, 14). To determine whether Rac3 could also cooperate with Raf to form foci, we cotransfected plasmids encoding activated Rac1 or Rac3 with a truncated and activated version of Raf into NIH 3T3 cells. We also cotransfected prenylation mutants of activated Rac1 or Rac3 with active Raf to determine whether the prenylation mutants could cooperate with Raf to support focus formation.

Consistent with its ability to activate c-Jun transcriptional transactivation like Rac1, Rac3 was also able to cooperate with Raf to
Farnesylated Rac Rescues Cells from GGTI-Mediated Inhibition of Anchorage-Independent Growth. If Rac proteins are physiologically relevant targets of GGTI, then a GGTI-resistant form of Rac should rescue GGTI-mediated inhibition of transformation. Due to the unexpected finding that GGTI inhibited the focus formation of Raf (Fig. 3C), we were unable to use the cooperative focus formation assay to answer this question. Instead, we turned to colony formation in soft agar, a transformed phenotype for which oncogenic Rac1 protein does not require coexpression of Raf (13). Therefore, we determined whether activated Rac3 alone could promote anchorage-independent growth in a soft agar colony-forming assay. As shown in Fig. 4, NIH 3T3 cells stably transfected with pCGN-hyg vector were not able to form colonies when suspended in soft agar. However, cells stably expressing activated Rac3 formed many colonies in soft agar, demonstrating that activated Rac3 alone could promote anchorage-independent growth.

To confirm that GGTI could disrupt soft agar colony formation by Rac3-expressing cells, we seeded NIH 3T3 cells expressing activated Rac3 and Rac3-GG onto soft agar in the presence of DMSO vehicle or 10 μM GGTI, a dose in which 97% of Rac1 and 83% and 89% of Rac3 and Rac3-GG, respectively, have been moved out of the membrane fraction as shown in Fig. 1B. As expected, GGTI totally ablated the ability of Rac3 and Rac3-GG to form colonies, whereas FTI did not (Fig. 4). This confirmed that Rac3-transforming activity is sensitive to GGTI but resistant to FTI and that Rac3 could be targeted by GGTI.

If Rac3 is a physiologically relevant target of GGTI, then a GGTI-resistant form of Rac3 should rescue GGTI-mediated inhibition of transformation. Therefore, we seeded NIH 3T3 cells stably expressing activated Rac3-F onto soft agar in the presence of DMSO, GGTI, or FTI. Overall, colony formation by Rac3-F was lower than that of Rac3-WT or Rac3-GG, but Rac3-F was able to overcome growth inhibition by GGTI to form colonies, whereas it formed no colonies in the presence of FTI (Fig. 4). Similar results were seen with activated Rac1 (data not shown).

Farnesylated Rac Rescues Cells from GGTI-Mediated Inhibition of Cell Spreading and Ruffling. To determine whether the membrane localization and membrane ruffling activity of Rac1 and Rac3 could be targeted by GGTIs, we transiently transfected activated versions of parental Rac1 and Rac3 and their prenylation mutants expressed from the vector pEGFP-C1 into NIH 3T3 cells. As can be seen in Fig. 5, in the presence of vehicle control, activated parental Rac1 and Rac3 and their prenylation mutants showed significant membrane ruffling and localization to the areas of ruffling with clear nuclear exclusion. However, in the presence of GGTI, parental Rac1 and Rac3 and the Rac3-GG mutant showed dramatically decreased ruffling activity and accumulation of Rac protein in the nucleus. Farnesylated versions of Rac1 and Rac3 were still able to ruffle in the presence of GGTI and had nuclear exclusion similar to vehicle control. However, farnesylated Rac1 and Rac3 were now sensitive to FTI, with decreased ruffling and increased accumulation in the nucleus. Activated Rac1- and Rac3-UN showed no ruffling activity and had diffuse localization throughout the cytoplasm and nucleus, much like pEGFP-C1 vector (Fig. 5; data not shown).

WT Rac3-F Rescues PDGF-Mediated Ruffling from GGTI Inhibition. Because mutationally activated versions of Rac proteins have not been identified in human tumors, it is thought that overexpression of WT forms or their hyperactivation may account for the contribution of Rac proteins to oncogenesis (31, 32). To determine whether Rac proteins are physiologically relevant targets for GGTIs, we used WT versions of Rac3 with parental and mutated prenylation sites in pEGFP-C1 to transfect Swiss 3T3 cells. Cells treated with DMSO, FTI, or GGTI were also treated with or without PDGF, a
known activator of Rac1, to determine whether subsequent membrane-ruffling activity was still subject to inhibition by GGTIs. As seen in Fig. 6, PDGF induces membrane ruffling and nuclear exclusion for WT Rac3 with either a parental (Rac3-P) or mutated prenylation site (Rac3-GG and Rac3-F). The ability to induce ruffling is inhibited for Rac3-P and Rac3-GG in the presence of GGTI, leading to flattened cells and an increase in nuclear localization. Ruffling still occurred in the presence of FTI in these cells. Cells transfected with farnesylated Rac3 were resistant to the inhibitory action of GGTI and had nuclear accumulation and the prevention of membrane ruffling only in the presence of FTI. Similar results were seen with WT Rac1 constructs (data not shown). These results indicate that both Rac1 and Rac3 are targets of GGTIs.

Fig. 3. Rac3 and prenylation mutants of both Rac1 and Rac3 are able to cooperate with Raf to transform NIH 3T3 cells in a focus formation assay. NIH 3T3 cells were transiently cotransfected with either pZIP vector only or pZIP containing an activated mutant of Raf, and either pCGN-hyg vector or pCGN-hyg constructs containing activated mutants of rac1 and rac3 with either the parental CAAX motif or motifs that were mutated to give the indicated prenylation mutants. Cells were fed with medium either containing no drugs (A) or with DMSO vehicle and 5 μM GGTI-2153 (C). A, after approximately 21 days of growth, plates were stained with crystal violet and foci were quantitated. Results are expressed as mean ± SD from duplicate plates. B, before staining, representative foci were photographed under a ×10 objective. C, crystal violet-stained plates demonstrate the negative effect of GGTI on Raf focus formation. Quantitation and photographs are representative of two to three independent experiments.
Rac3 can mediate PDGF responsiveness and that GGTI-resistant Rac proteins can overcome GGTI inhibition of a physiologically important function of WT Rac proteins.

DISCUSSION

Rac1 proteins are involved in membrane ruffling, morphological and growth transformation, and signaling to a variety of downstream transcription factors, including c-Jun, SRF, and nuclear factor κB (1, 33, 34). Considerably less is known about the consequences of Rac3 activation. Rac3 has been reported to activate JNK (15) and PAK (17) and to up-regulate DNA synthesis in a PAK-dependent, JNK-independent manner (17). Rac3 GTP levels have been reported to be elevated in breast cancer cell lines and primary tumor tissues (17). These results, along with the sequence similarity of Rac3 and Rac1, suggest that Rac3 could also have transforming activity. In support of this idea, we show here that constitutively activated mutant forms of Rac3 cause both focus formation and growth in soft agar in NIH 3T3 cells, in a manner very similar to that of Rac1. Additionally, we show here that both WT Rac1 and Rac3 induce membrane ruffling in response to PDGF. Thus, Rac3 is functionally similar to Rac1 in terms of responsiveness to growth factor stimulation and transforming ability.

Our data from the experiments outlined above suggest that Rac1 and Rac3 function similarly. Although there is substantial sequence identity in the classical effector domain region of these highly related proteins, other important elements of sequence divergence exist between Rac1 and Rac3 (15). For example, differences exist in and around the Rho insert domain, a sequence that is unique to Rho family proteins (35), and in the hypervariable region at the COOH terminus that could dictate functional distinctions. Furthermore, the early emergence of Rac1 and Rac3 in evolution (36) suggests that functional distinctions must exist. Amino acids in and around the Rho insert region of Rac1 and other Rho family members are known to contribute to effector binding (8, 37–39). Possible distinctions between Rac1 and Rac3 may also lie in the COOH-terminal hypervariable domain, a region that dictates isoprenoid modification and is important for membrane localization of small GTPases and for biological activity (18, 40, 41).

Rac1 is known to be modified by a GG isoprenoid lipid (25). The CAX motif of Rac3, CTVF, with F in the X position, suggests that it could be a potential target for either GGTase I or FTase (26). Our results demonstrate that Rac3 is likely to be mostly geranylgeranylated in cells. Individual small GTPases may differ in their requirement for modification by a specific isoprenoid moiety for function. For example, WT H-Ras is growth inhibitory only when modified by a GG group instead of its native F group (42), but the biological activity of activated farnesylated RhoA is indistinguishable from that of the authentically geranylgeranylated RhoA (27). We have shown here that both oncogenic and WT Rac1 and Rac3 appear to be tolerant of modification by either a GG group or F group for transformation and membrane-ruffling activities. Thus, the consequences of alternate lipid modification of Rac1 and Rac3 are more similar to what has been shown for activated RhoA than for WT H-Ras, suggesting that farnesylated Rac proteins are useful tools to investigate whether Rac1 and Rac3 are physiological targets for inhibition of GGTase I by GGTIs, the basis for a novel anticancer therapy.

FTase, the enzyme that attaches the F group to Ras, RhoB, and a subset of other small GTPases, has long been a target for rational drug design (19, 20, 43–45). FTIs are in Phase I–III clinical trials for anticancer treatment, although the identity of the most critical targets...
that can explain FTI antitumor activity are still under investigation. GGTase I modifies many proteins in the Rho family of small GTPases, including Rac1 and Rac3, by attaching a GG group. GGTase I has also recently become a target for rational drug design, with the development of new inhibitors to block geranylgeranylation (19, 20). GGTIs have been shown to arrest human tumor cell growth in vitro (21, 22) and to reduce tumor growth in animal models (23), yet the physiologically relevant downstream targets of GGTIs have

Fig. 5. Farnesylated Rac rescues cells from GGTI-mediated inhibition of cell spreading and ruffling. NIH 3T3 cells plated on coverslips were transiently transfected with pEFGFP-C1 constructs containing either activated mutants of rac1 with a parental CAAX motif or with motifs mutated to express F-modified or UN Rac1 (A) or activated mutants of rac3 with a parental CAAX motif or with motifs mutated to express GG- or F-modified or UN Rac3 (B). Immediately after transfection, cells were placed in medium containing DMSO vehicle, FTI, or GGTI and allowed to express protein for 48 h. Live cells expressing green fluorescent protein-tagged Rac1 and Rac3 proteins were viewed with a fluorescent microscope and photographed under a ×20 objective. Photographs shown are representative of four experiments.
not been determined. Candidate downstream targets include the geranylgeranylated members of Rho family GTPases.

Inhibition of RhoA by GGTI led to an increase in p21waf1/cip1 expression, which is normally repressed by RhoA (46). This may help to mediate the G1 arrest that is seen with GGTI treatment (21, 22). However, farnesylated RhoA, although functionally equivalent to the native geranylgeranylated RhoA, is unable to restore RhoA activity in the presence of GGTI (27). These results suggest that although RhoA may be an important and necessary target of GGTIs, it may not be sufficient to mediate the inhibitory action of GGTIs.

We used the GGTI-insensitive, farnesylated versions of Rac1 and Rac3 to determine whether Rac proteins are biologically important.

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Fig. 6. WT Rac3-F rescues PDGF-mediated ruffling from GGTI inhibition. Swiss 3T3 cells plated on coverslips were transiently transfected with pEGFP-C1 constructs containing WT rac3 with a parental CAAX motif (A), WT rac3 with a CAAX motif mutated to express GG-modified protein (B), or WT rac3 with a CAAX motif mutated to express F-modified protein (C). After transfection, cells were grown continually in the presence of DMSO vehicle, FTI, or GGTI for 24 h in complete medium and for an additional 24 h in serum-free medium. Cells were then treated for 20 mm with vehicle or with 20 ng/ml PDGF, viewed with a fluorescent microscope, and photographed under a ×20 objective. Results are representative of two independent experiments.
downstream targets for GGTIs. If a GGTI-insensitive form of Rac can rescue cells from GGTI-mediated growth inhibition, then Rac is likely to be either an important mediator of that inhibition or downstream of a critical GGTase I target. Therefore, we investigated the ability of farnesylated forms of oncogenic Rac1 and Rac3, which we demonstrated to be GGTI-insensitive, to rescue cells from GGTI-mediated inhibition of anchorage-independent growth and from inhibition of membrane-ruffling activity. These results suggested that Rac proteins may play an important role in the cellular response to GGTIs. However, oncogenically mutated forms of Rac and Rho proteins have not been found in human cancer cells; instead, it is thought that amplification of Rho family proteins or activation of their upstream regulators such as exchange factors contribute to the ability of these GTPases to influence the transformed phenotype (31, 32). Therefore, we also investigated whether a WT version of farnesylated Rac was resistant to the effects of GGTI. Farnesylated versions WT of Rac1 and Rac3 were both resistant to the effects of GGTI and continued to form ruffles after PDGF stimulation. These results suggest that in a physiological setting such as a human tumor, in which Rac proteins are unlikely to contain activating mutations, Rac proteins can still be functionally targeted by GGTIs.

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